A Single Nucleotide Polymorphism in the 5′ Untranslated Region of *RAD51* and Risk of Cancer among *BRCA1/2* Mutation Carriers[1]

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Abstract

RAD51 colocalizes with both BRCA1 and BRCA2, and genetic variants in *RAD51* would be candidate *BRCA1/2* modifiers. We searched for *RAD51* polymorphisms by sequencing 20 individuals. We compared the polymorphism allele frequencies between female *BRCA1/2* mutation carriers with and without breast or ovarian cancer and between population-based ovarian cancer cases with *BRCA1/2* mutations to cases and controls without mutations. We discovered two single nucleotide polymorphisms (SNPs) at positions 135 g→c and 172 g→t of the 5′ untranslated region. In an initial group of *BRCA1/2* mutation carriers, 14 (21%) of 67 breast cancer cases carried a "c" allele at *RAD51*:135 g→c, whereas 8 (7%) of 119 women without breast cancer carried this allele. In a second set of 466 mutation carriers from three centers, the association of *RAD51*:135 g→c with breast cancer risk was not confirmed. Analyses restricted to the 216 *BRCA2* mutation carriers, however, showed a statistically significant association of the 135 "c" allele with the risk of breast cancer (adjusted odds ratio, 3.2; 95% confidence limit, 1.4–40). *BRCA1/2* mutation carriers with ovarian cancer were only about one half as likely to carry the *RAD51*:135 g→c SNP. Analysis of the *RAD51*:135 g→c SNP in 738 subjects from an Israeli ovarian cancer case-control study was consistent with a lower risk of ovarian cancer among *BRCA1/2* mutation carriers with the "c" allele. We have identified a *RAD51* 5′ untranslated region SNP that may be associated with an increased risk of breast cancer and a lower risk of ovarian cancer among *BRCA2* mutation carriers. The biochemical basis of this risk modifier is currently unknown.

Introduction

Genetic factors are important in breast and ovarian cancer, but less than 10% are attributable to the inheritance of mutations in a single gene, such as *BRCA1* and *BRCA2* (1). Although relatively uncommon, female carriers of mutations in the *BRCA1* and *BRCA2* genes are estimated to have breast cancer risks of 37% to over 85% by age 70 and ovarian cancer risks approximately one half as high (2–5). Both *BRCA1* and *BRCA2* appear to confer similarly high risks of female breast cancer, whereas ovarian cancer risk is higher for *BRCA1* mutation carriers and male breast cancer risk is largely confined to *BRCA2* carriers (1, 6). The heterogeneity of risk in breast and ovarian cancer between different families segregating mutations in the same gene and the estimated average risk of breast cancer of about 50% both suggest the existence of important environmental or genetic modifiers of risk. Some potential genetic modifiers have been identified, including rare polymorphisms in H-*ras* for ovarian cancer (7) and variation in the androgen receptor gene for breast cancer risk (8).

The precise mechanisms by which mutations in *BRCA1/2* predispose to breast and ovarian cancer are unknown. They are large proteins that share little sequence similarity with each other or with other known sequences. Both appear to be involved in double-strand DNA break repair, particularly because of their association with RAD51 (9). RAD51 is a homologue of the *Escherichia coli* recA protein and is involved in recombination and the repair of double-strand DNA breaks (10). RAD51 shares 60% protein identity with the yeast scRad51, which has been studied extensively (10, 11). The relative consistency of the findings of BRCA1 and BRCA2 associating with RAD51, their possible complementary roles as DNA damage repair proteins, and the availability of a model organism system in which to investigate findings led us to consider *RAD51* as a candidate modifier locus.

Materials and Methods

Polymorphisms in *RAD51* were investigated by complete sequence analysis of the gene in 10 individuals who had devel-

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oped both breast cancer and at least one other primary cancer obtained from a national cohort of radiological technologists participating in a prospective epidemiological study (12), and the 5′UTR[7] was sequenced in an additional 10 individuals (subjects 01 and 02 from each of the Center d'Etude du Polymorphisme Humain families 1331, 1332, 1347, 1362, and 1413; Coriell Institute, Camden, NJ). Sequencing these subjects revealed a g→c polymorphism at position 135 and a g→t at position 172 of the published cDNA (GenBank accession no. D14134.1)

Two groups were studied to test for allelic association, including: (*a*) a series of female *BRCA1/2* mutation carriers; and (*b*) Jewish subjects from an Israeli ovarian cancer case-control study. All of the subjects with individually identifiable samples gave informed consent for participation. Study group (*a*) included all of the known female *BRCA1* and/or *BRCA2* heterozygous mutation carriers from each of four study centers. Disease associations for the two SNPs were first assessed in two groups of subjects from the NCI. One group was from the Washington Ashkenazi Study, a community-based sample of 86 females who carried the185delAG or 5382 insC mutations in *BRCA1* and/or the 6174delT mutation in *BRCA2* (3). The second NCI group consisted of 100 female members of 27 breast/ovarian cancer families with identified *BRCA1* or *BRCA2* mutations from the NCI Genetic Epidemiology Branch family registry (13).

To replicate the findings with *RAD51*:135 g→c, study group (*a*) was expanded to include subjects from three additional centers. Subjects from the Fox Chase Cancer Center were recruited from high-risk and breast cancer clinics at the Fox Chase Cancer Center and affiliated hospitals in the Delaware Valley, as well as through self- and physician-referral. Subjects from Australia were either members of multiple-case families ascertained in family cancer clinics by the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (14) or the South Australian Clinical Genetics Service or from the population-based case-control-family studies of the Australian Breast Cancer Family Study/Cooperative Family Registry for Breast Cancer Studies (15) and the Australian Jewish Breast Cancer Study (16). All of the Jewish *BRCA1/2* mutation carriers ascertained from Memorial Sloan Kettering Cancer Center were also studied.

Study group (*b*) included a subset of subjects from a case-control study of all of the cases of invasive epithelial ovarian cancer diagnosed among Jews in Israel between 1994–1999 and age- and ethnicity-matched population controls tested for the three founder *BRCA1/2* mutations (17, 18). For study group (*b*), *RAD51*:135 g→c SNP typing was completed for 213 (86%) of 249 cases who were *BRCA1/2* mutation positive and for random subsets of 304 of the 598 mutation-negative cases and 221 of the 777 mutation-negative controls from this study.

Most *BRCA1/2* mutations were identified in the laboratories of the individual study centers using a number of techniques, whereas some were identified by complete sequencing performed at Myriad Genetics Laboratories (Salt Lake City, UT). We were relatively conservative in our definition of mutations, because only two of the 73 included in this study were *BRCA1* missense mutations (C61G, observed to cosegregate with disease in multiple families worldwide, and V1713A, an extremely rare variant in a family with a logarithm of the odds score of 1.3 for linkage to *BRCA1*). The remaining mutations were nonsense, frameshift, splice junction, or large genomic rearrangements. Among group (*a*) subjects, the two most common mutations were *BRCA1*:185delAG ($n = 216$) and *BRCA2*:6174delT ($n = 124$).

Laboratory Analysis. To identify *RAD51* polymorphisms, because the genomic structure of *RAD51* was unpublished, we extracted total RNA from lymphoblastoid cell lines, performed reverse transcription-PCR, and sequenced the cDNA in overlapping fragments (conditions and primer sequences available on request). The two 5′UTR SNPs identified could be detected in one single-strand conformational polymorphism/heteroduplex analysis assay using genomic DNA as template and the primers 21F (5′-ccgagccctaaggagagtgcggcgcttc-3′) and 232R (5′-tactcggtccgcagcgctcctctctccag-3′). Thirty-five samples were sequenced and analyzed with single-strand conformational polymorphism/heteroduplex analysis to verify the correlation between the observed banding patterns and the sequences at the two SNPs. Over 97% of the samples could be scored for both loci by blinded review by two individuals. The *RAD51*:135 g→c SNP was assayed in some subjects using a standard PCR and *Bst*NI digestion specific for the wild-type "g" allele, using the primers 64F (5′-gggaactgcaactcatctgggtt) and 170R (5′-tgg cac gcg ccc gac) at 200 nM concentration to amplify a 107-bp product. The population allele frequencies for the polymorphisms were estimated by genotyping 40 unrelated Ashkenazi spouses and 62 non-Jewish spouses from the NCI registry families and 79 males from the Washington Ashkenazi Study. The *RAD51*:135c allele frequency was 0.05 among Jews and 0.07 among non-Jews, and the *RAD51*:172t allele frequencies were 0.43 and 0.41, respectively. The genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium.

Statistical Analysis. To test for association between the *RAD51*:135 g→c SNP and cancer risk among the series of 652 female *BRCA1/2* mutation carriers (study group *a*) for breast cancer analyses, the 345 subjects who had been diagnosed with breast cancer were classified as cases and the remaining 307 subjects as controls. Because analyses of ovarian cancer risk were undertaken after those for breast cancer, we excluded all of the subjects who had developed breast cancer, leaving 44 subjects with ovarian cancer and 263 control subjects with neither breast nor ovarian cancer. A subject's age was taken as the age at diagnosis for cases and the current age, age at bilateral preventive surgery (mastectomy or oophorectomy), or age at death for controls. The ORs comparing SNP carrier frequency between those with and without cancer were obtained using logistic regression models. All of the analyses were adjusted for age, ethnicity (Jewish, non-Jewish), gene (*BRCA1* or *BRCA2*), and study center, although the estimates changed very little with adjustment. Because observations from related subjects may not be independent, adjusted CLs for all of the ORs and *P*s were calculated using a bootstrap resampling technique. Two thousand resamplings of the data, grouped on family, were drawn with replacement (19). The 2.5% and 97.5% empirical values of the regression coefficients were used as the 95% CLs, and the significance level was calculated as twice the proportion of replicates that resulted in a regression coefficient less than zero.

For subjects from the ovarian cancer case-control study (study group *b*), we compared the prevalence of the *RAD51*:135 g→c SNP between *BRCA1/2* mutation-positive cases *versus* all of the others with a Mantel-Haenszel OR, adjusted for ethnicity (Ashkenazi/non-Ashkenazi). All of the statistical analyses were performed using S-Plus 2000 Professional (Mathsoft, Inc.).

[7] The abbreviations used are: UTR, untranslated region; SNP, single-nucleotide polymorphism; NCI, National Cancer Institute; OR, odds ratio; CL, confidence limit.

Table 1 Characteristics of female BRCA1 and BRCA2 heterozygous mutation carriers

Study center	Total no. subjects	No. (%) BRCA1-positive	No. (%) BRCA2-positive[a]	No. (%) Ashkenazi Jewish	No. (%) with breast cancer[b]	Mean age (range) at breast cancer	No. (%) with ovarian cancer[b]	Mean age (range) at ovarian cancer	No. of unaffected subjects[c]	Mean age (range) of unaffected subjects	No. of families
NCI Genetic Epidemiology Branch	100	76 (76)	24 (24)	21 (21)	41 (41)	42 (22–69)	15 (15)	48 (35–64)	47	46 (23–87)	27
Washington Ashkenazi Study	86	47 (55)	39 (45)	86 (100)	25 (29)	43 (27–69)	3 (3)	49 (47–53)	60	46 (26–82)	84
Australia	171	92 (54)	79 (46)	40 (23)	104 (61)	40 (23–68)	10 (6)	53 (36–75)	59	44 (20–74)	102
Fox Chase Cancer Center	149	116 (78)	33 (22)	107 (72)	72 (48)	42 (26–67)	29 (19)	51 (32–65)	59	38 (19–73)	93
Memorial Sloan Kettering Cancer Center	146	105 (72)	41 (28)	146 (100)	102 (70)	41 (25–77)	15 (10)	49 (38–61)	38	43 (23–78)	119
Total	652	436 (67)	216 (33)	400 (61)	344 (53)	41 (22–77)	72 (11)	50 (32–75)	263	44 (19–87)	425

[a] The five subjects who are heterozygous for both a *BRCA1* and *BRCA2* mutation are categorized as *BRCA2* mutation carriers.
[b] Includes 28 subjects with both breast and ovarian cancer.
[c] Subjects who have not had breast or ovarian cancer.

Results

By complete sequencing of *RAD51* cDNA in 10 individuals, no differences were observed in the amino acid-coding portions of *RAD51* (compared with GenBank accession no. D14134.1), but we observed a polymorphism in the 5′UTR at nucleotide position 172 g→t. To analyze this polymorphism in our sample of female *BRCA1/2* mutation carriers, in which only genomic DNA was available for most, we developed a genomic PCR-based assay that upon sequencing in 10 unrelated individuals from the Center d'Etude du Polymorphisme Humain collection revealed another, less common 5′UTR variant nucleotide at position 135 g→c (GenBank accession no. AF233739.1).

To test for association between the SNPs and cancer risk, we compared the frequencies between female *BRCA1/2* mutation carriers with and without breast and/or ovarian cancer with the characteristics shown in Table 1. Approximately one half of the subjects had breast cancer and approximately one third carried a *BRCA2* mutation. The average number of subjects/family ranged from one in the Washington Ashkenazi Study group to 3.7 for the Genetic Epidemiology Branch families. In the hypothesis-generating data from NCI, 14 (21%) of the 67 female *BRCA1/2* mutation carriers who had developed breast cancer carried at least one *RAD51*:135c allele, whereas 8 (7%) of 119 subjects without breast cancer carried a "c" allele (adjusted OR, 3.1; 95% CL, 1.1–14; $P = 0.04$). There was no difference in the frequency of the three genotypes at *RAD51*: 172 g→t between breast cancer cases (14% t/t; 49% g/t) and controls (18% t/t; 38% g/t).

We sought to replicate the association by determining the *RAD51*:135 g→c genotypes of an additional 466 *BRCA1/2* mutation carriers from three study locations. The frequency of the *RAD51*:135c was only slightly higher among breast cancer cases (12%) *versus* subjects without breast cancer (10%; adjusted OR, 1.3; 95% CL, 0.7–2.8; $P = 0.3$). Combining all of the subjects, 13% of breast cancer cases carried a *RAD51*:135 "c" allele *versus* 9% of controls, with an adjusted OR of 1.6 (95% CL, 1.0–3.4; $P = 0.06$). If we exclude subjects with ovarian cancer in this comparison, the frequency of *RAD51*: 135c was 43 of 317 (14%) among breast cancer cases *versus* 25 of 263 (10%) among women without cancer. The *RAD51*:172 g→t SNP was tested in all of the subjects except those from Australia and was again not associated with cancer risk, with observed allele frequencies of 0.41 *versus* 0.38 among *BRCA1*-positive breast cancer cases *versus* those without breast cancer and 0.38 *versus* 0.37 among BRCA2-positive subjects.

The combined data set had sufficient numbers of subjects to perform analyses restricted to subsets of the data (Table 2). There was a statistically significant increased risk of breast cancer associated with the *RAD51*:135c allele among the 216 *BRCA2* mutation carriers (OR, 3.2; 95% CL, 1.4–40; $P = 0.01$). The median age at breast cancer diagnosis among *BRCA2* mutation carriers and at least one *RAD51*:135c allele was 37 years *versus* 41 years for those without the SNP ($P = 0.02$; two-sided nonparametric median test; Ref. 20). The magnitude of the risk was strongest for the two study groups in which all of the subjects were Jewish (Washington Ashkenazi Study and Memorial Sloan Kettering Cancer Center). In analyses restricted to the 400 Jewish subjects, an increased risk was present for *BRCA1* and *BRCA2* mutation carriers combined, with 13% of breast cancer cases carrying the *RAD51*:135c *versus* 7% without breast cancer (OR, 2.4; 95% CL, 1.2–5.8; $P = 0.02$). The associations among the Jewish subjects were relatively consistent across study centers and were stronger for *BRCA2* mutation carriers, but small sample sizes precluded reliable statistical testing of subgroups.

When considering the entire group of female *BRCA1/2* mutation carriers, there were sufficient numbers of subjects with ovarian cancer to compare the *RAD51*:135 g→c SNP allele frequencies. But because these analyses were undertaken after the increased SNP frequency was observed among breast cancer cases, we excluded women who had been diagnosed with breast cancer. Ovarian cancer cases were only about one half as likely to carry at least one *RAD51*:135c allele (2 of 44; 5%) compared with controls (25 of 263; 10%; OR, 0.4; 95% CL, 0.1–1.5; $P = 0.3$). If breast cancer cases were not excluded, 5 of 72 (7%) ovarian cancer cases carried at least one *RAD51*: 135c allele compared with 68 of 580 (12%) controls.

The suggestion of a lower risk of ovarian cancer led us to determine the *RAD51*:135 g→c SNP genotype in a completely different study setting. Among a large group of invasive epithelial ovarian cancer cases and controls from Israel, the *RAD51*:135c allele was present in 6.5% of 62 *BRCA2* mutation-positive cases, 10% of 151 *BRCA1* mutation-positive cases, 13% of 304 *BRCA1/2*-negative cases, and 14% of 221 controls (Table 3). The OR associated with the *RAD51*:135c allele, adjusted for ethnicity, was 0.66 (95% CL, 0.4–1.15) for *BRCA1/2*-positive cases *versus* all of the other subjects.

Discussion

We did not observe any sequence variation in the amino acid-coding portion of the *RAD51* gene among 10 individuals completely analyzed, which is consistent with another report (21). Although an amino acid variant has been identified in two

Table 2 Association between *RAD51:*135g→c SNP and breast cancer risk among BRCA1 and BRCA2 heterozygous mutation carriers

Study center	*RAD51: 135g→c* genotype	No. (%) with genotype							
		BRCA1 Mutation Carriers				*BRCA2* Mutation Carriers			
		Breast cancer	Ovarian cancer	Both breast and ovarian cancer	Neither cancer	Breast cancer	Ovarian cancer	Both breast and ovarian cancer	Neither cancer
Initial observation									
NCI Genetic Epidemiology Branch	gc + cc	6 (21)	0 (0)	0 (0)	5 (14)	4 (44)	0 (0)	0 (0)	1 (10)
	gg	23 (79)	8 (100)	2 (100)	32 (86)	5 (56)	3 (100)	2 (100)	9 (90)
Washington Ashkenazi Study	gc + cc	2 (15)	0 (0)	0 (0)	2 (6)	2 (20)	0	0	0 (0)
	gg	11 (85)	1 (100)	2 (100)	29 (94)	8 (80)	0	0	29 (100)
NCI total[a]	gc + cc	8 (19)	0 (0)	0 (0)	7 (10)	6 (32)	0 (0)	0 (0)	1 (3)
	gg	34 (81)	9 (100)	4 (100)	61 (90)	13 (68)	3 (100)	2 (100)	38 (97)
Replication sets									
Australia	gc + cc	5 (10)	0 (0)	0 (0)	2 (6)	6 (12)	0 (0)	0	3 (11)
	gg	47 (90)	6 (100)	2 (100)	30 (94)	44 (88)	2 (100)	0	24 (89)
Fox Chase Cancer Center	gc + cc	8 (19)	1 (8)	2 (20)	10 (20)	2 (11)	0 (0)	0 (0)	1 (13)
	gg	34 (81)	12 (92)	8 (80)	41 (80)	17 (89)	5 (100)	1 (100)	7 (88)
Memorial Sloan Kettering Cancer Center	gc + cc	4 (6)	1 (20)	1 (11)	1 (4)	4 (13)	0 (0)	0	0 (0)
	gg	59 (94)	4 (80)	8 (89)	27 (96)	26 (87)	1 (100)	0	10 (100)
Replication set total[b]	gc + cc	17 (11)	2 (8)	3 (14)	13 (12)	12 (12)	0 (0)	0 (0)	4 (9)
	gg	140 (89)	22 (92)	18 (86)	98 (88)	87 (88)	8 (100)	1 (100)	41 (91)
Total (all of the centers)	gc + cc	25 (13)	2 (6)	3 (12)	20 (11)	18 (15)	0 (0)	0 (0)	5 (6)
	gg	174 (87)	31 (94)	22 (88)	159 (89)	100 (85)	11 (100)	3 (100)	79 (94)

[a] OR = 3.1 (95% CL 1.1–14) associated with carrying at least one *RAD51:*135 "c" allele, adjusted for age, ethnicity, gene, and study center, comparing subjects with breast cancer *versus* those without breast cancer; BRCA1/BRCA2 mutation carriers combined.
[b] OR = 1.3 (95% CL 0.7–2.8) associated with carrying at least one *RAD51:*135 "c" allele, adjusted for age, ethnicity, gene, and study center, comparing subjects with breast cancer *versus* those without breast cancer; BRCA1/BRCA2 mutation carriers combined.

Table 3 Comparison of *RAD51:*135g→c SNP genotypes among subjects in National Israeli Study of Ovarian Cancer[a]

Ethnicity	RAD51-135g→c SNP genotype	No. (%) with each genotype			
		BRCA1 mutation-positive cases	*BRCA2* mutation-positive cases	*BRCA1/2* mutation-negative cases	*BRCA1/2* mutation-negative controls
Ashkenazi	gg	118 (90)	52 (93)	180 (87)	128 (89)
	gc + cc	13 (10)	4 (7)	26 (13)	16 (11)
	Total	131 (100)	56 (100)	206 (100)	144 (100)
Non-Ashkenazi	gg	18 (90)	6 (100)	83 (85)	62 (81)
	gc + cc	2 (10)	0 (0)	15 (15)	15 (19)
	Total	20 (100)	6 (100)	98 (100)	77 (100)

[a] Mantel-Haenszel OR, adjusted for ethnicity, comparing the SNP between *BRCA1/2* mutation-positive cases *versus* all of the others = 0.66 (95% CL, 0.4–1.15).

Japanese patients with breast cancer (22), it appears that common amino acid variants in *RAD51* are not cancer susceptibility alleles. We did observe two SNPs at nucleotide positions 135 g→c and 172 g→t in the 5′UTR of the gene. The 135 g→c SNP was present in approximately 14% of non-Jewish control individuals in the United States and in approximately 10% of Jewish controls from the United States. On the basis of the frequency of the 135 g→c allele among population-based controls from the ovarian cancer case-control data from Israel presented here, it appears that the SNP is more common in non-Ashkenazi Jews in Israel, because approximately 19% of controls were heterozygous carriers. All of the subjects who were homozygous 135c/c were also homozygous 172 g/g, and among heterozygous 135 g/c individuals, where phase could be determined, the 135c occurred only on the 172 g chromosome, suggesting that the SNPs are in linkage disequilibrium.

We evaluated the two *RAD51* SNPs as potential modifying alleles, initially in a series of 186 female *BRCA1/2* heterozygous mutation carriers from NCI. Because of the relatively small sample size, we combined *BRCA1* and *BRCA2* mutation carriers and observed a statistically significant association between the risk of breast cancer and carrying the rarer "c" allele at nucleotide 135 of the *RAD51* gene. Female carriers of either a *BRCA1* or a *BRCA2* mutation who also carried this SNP had about 3-fold increased odds of developing breast cancer compared with *BRCA1/2* mutation carriers without the SNP. We attempted to replicate this finding by determining the *RAD51*: 135 g→c SNP in an additional set of 446 female *BRCA1/2*

mutation carriers from three study centers. Analyzing *BRCA1/2* mutation carriers together, the association was not confirmed, with the *RAD51*:135c allele nearly equally frequent among women with and without breast cancer.

The entire group of 652 BRCA1/2 mutation carriers was large enough to conduct analyses of subgroups, and we analyzed *BRCA1* and *BRCA2* mutation carriers separately. In the hypothesis-generating data set from NCI, the association was stronger among *BRCA2* mutation carriers. In the replication sets, although there was clearly no association among the *BRCA1* mutation carriers, more *BRCA2* mutation carriers with breast cancer (12%) carried the 135c SNP compared to those without breast cancer (8%). In the entire group of 216 *BRCA2* mutation carriers studied, carrying the *RAD51*:135c allele was associated with a significant risk of breast cancer (OR, 3.2; 95% CL, 1.4–40). Because only nonsense, frameshift, or splicing *BRCA2* mutations were studied, 26% of the studied mutations would be expected to have lost all of the eight BRC repeats, the regions of *BRCA2* involved in the *RAD51* interaction (23), whereas an additional 69%, including the common 6174delT mutation, would be predicted to have lost at least some of the terminal BRC repeats.

We observed the opposite effect with regard to ovarian cancer risk for the *RAD51*:135 g→c SNP. None of the 11 *BRCA2* mutation-positive subjects with ovarian cancer (and no breast cancer) carried the *RAD51*:135c allele, compared with 6% of 84 subjects with neither breast nor ovarian cancer. The *RAD51*:135c allele was also less common in these two groups of *BRCA1* mutation carriers, present in 6% of 33 ovarian cancer cases *versus* 13% of controls. This apparent lower risk of ovarian cancer was explored further in an entirely different study population. Among 731 subjects from the National Israeli Study of Ovarian Cancer characterized as to *BRCA1/2* founder mutations and *RAD51*:135 g→c SNP genotypes, *BRCA1/2* mutation-positive ovarian cancer cases were 34% less likely to carry the *RAD51*:135c allele compared with *BRCA1/2* mutation-negative cases and controls, with *BRCA2* mutation-positive cases the least likely to carry the SNP. Although not formally statistically significant, the size of the effect was similar to that predicted from the hypothesis-generating observation in the series of female *BRCA1/2* mutation carriers.

The populations of *BRCA1/2* mutation carriers we studied are not ideal, but given the low frequency of mutations among the general population and the technical difficulties of detecting mutations, optimal epidemiological designs to identify factors that may modify mutation carriers' cancer risk require inordinately large sample sizes. The female *BRCA1/2* mutation carriers consisted predominantly of members of families initially identified in high-risk clinical settings. Almost all of the unaffected mutation carriers were relatives of cancer cases in whom the mutation was initially identified. All of the Washington Ashkenazi Study participants volunteered for a single cross-sectional study. Breast cancer "cases," therefore, were survivors who volunteered after a variable length of time since their diagnosis and were not a random or epidemiological sampling of incident cases. If survival is different between individuals with different *RAD51* SNP genotypes, this could lead to differential participation and bias the results. Survival bias should be somewhat less of a problem in the largely family-based study groups, which are followed prospectively and include both living and deceased individuals.

Is the *RAD51*:135 g→c SNP association with cancer risk real? The statistical evidence from this study is not overwhelming, with the initial observation, made by analyzing a combined group *BRCA1* and *BRCA2* mutation carriers, not confirmed in a replication set of over 400 *BRCA1/2* mutation carriers. But the more consistent association across study sites for *BRCA2* mutation carriers, the supportive findings in an entirely different epidemiological study group from the National Israeli Ovarian Cancer Study, and the replication of the association among *BRCA2* mutation carriers by an independent group, in which 17% of 46 mutation carriers with breast or ovarian cancer from Israel carried the *RAD51*:135c allele compared with 5% of 41 healthy carriers (24), suggests that it is.

Biochemical evidence as to how this SNP might affect cancer risk would greatly bolster the association but is lacking. Although there are some known regulatory elements associated with 5′UTRs (25, 26), it is not immediately clear how the SNPs identified in this study might affect gene function. One possibility is that it affects mRNA splicing, but we did not observe consistent, alternate reverse transcription-PCR products that correlated with the SNP. Other mechanisms, such as regulation of transcription (*e.g.*, through methylation), translation (*e.g.*, through internal ribosomal entry segments), or mRNA stability, remain to be studied. This polymorphism could also be in linkage disequilibrium with another sequence change in a regulatory region of the *RAD51* gene or with another nearby gene that affects the incidence of breast cancer in *BRCA1/2* mutation carriers. Studies correlating the presence of the polymorphism with *RAD51* expression and function will be needed to support the statistical association we observed.

The *RAD51*:135 g→c SNP was associated with an increased risk of breast cancer and a lower risk of ovarian cancer among the series of female *BRCA1/2* mutation carriers studied. This divergence in findings may be attributable to chance or may reflect different biological effects in the two tissues. The differences may also be attributable to the fact that to a large extent breast and ovarian cancer are competing end points. If *RAD51* is primarily active in one tissue, *e.g.*, if the SNP is associated with an increased risk of breast cancer, fewer polymorphism carriers would then survive to be diagnosed with ovarian cancer, and one might expect to see opposite associations for the two end points. It is unknown whether the *RAD51*:135 g→c SNP is also associated with an increased breast cancer risk among the majority of women who do not carry *BRCA1/2* mutations.

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References

1. Ford, D., Easton, D. F., Stratton, M., Narod, S., Goldgar, D., Devilee, P., Bishop, D. T., Weber, B., Lenoir, G., Chang-Claude, J., Sobol, H., Teare, M. D., Struewing, J., Arason, A., Scherneck, S., Peto, J., Rebbeck, T. R., Tonin, P., Neuhausen, S., Barkardottir, R., Eyfjord, J., Lynch, H., Ponder, B. A., Gayther,

S. A., and Zelada-Hedman, M., and The Breast Cancer Linkage Consortium. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. Am. J. Hum. Genet., *62:* 676–689, 1998.

2. Easton, D. F., Ford, D., and Bishop, D. T. The Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in *BRCA1*-mutation carriers. Am. J. Hum. Genet., *56:* 265–271, 1995.

3. Struewing, J. P., Hartge, P., Wacholder, S., Baker, S. M., Berlin, M., McAdams, M., Timmerman, M. M., Brody, L. C., and Tucker, M. A. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. N. Engl. J. Med., *336:* 1401–1408, 1997.

4. Thorlacius, S., Struewing, J. P., Hartge, P., Olafsdottir, G. H., Sigvaldason, H., Tryggvadottir, L., Wacholder, S., Tulinius, H., and Eyfjord, J. E. Population-based study of risk of breast cancer in carriers of *BRCA2* mutation. Lancet, *352:* 1337–1339, 1998.

5. Hopper, J. L., Southey, M. C., Dite, G. S., Jolley, D. J., Giles, G. G., McCredie, M. R. E., Easton, D. F., and Venter, D. J. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein- truncating mutations in *BRCA1* and *BRCA2*. Cancer Epidemiol. Biomark. Prev., *8:* 741–747, 1999.

6. Narod, S. A., Ford, D., Devilee, P., Barkardottir, R. B., Lynch, H. T., Smith, S. A., Ponder, B. A., Weber, B. L., Garber, J. E., Birch, J. M., Cornelis, R. S., Kelsell, D. P., Spurr, N. K., Smyth, E., Haites, N., Sobol, H., Bignon, Y. J., Chang-Claude, J., Hamann, U., Lindblom, A., Borg, A., Piver, M. S., Gallion, H. H., Struewing, J. P., Whittemore, A. S., Tonin, P., Goldgar, D. E., and Easton, D. F. An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. Breast Cancer Linkage Consortium. Am. J. Hum. Genet., *56:* 254–264, 1995.

7. Phelan, C. M., Rebbeck, T. R., Weber, B. L., Devilee, P., Ruttledge, M. H., Lynch, H. T., Lenoir, G. M., Stratton, M. R., Easton, D. F., Ponder, B. A., Cannon, A. L., Larsson, C., Goldgar, D. E., and Narod, S. A. Ovarian cancer risk in *BRCA1* carriers is modified by the *HRAS1* variable number of tandem repeat (*VNTR*) locus. Nat. Genet., *12:* 309–311, 1996.

8. Rebbeck, T. R., Kantoff, P. N., Krithivas, K., Neuhausen, S., Blackwood, M. A., Godwin, A. K., Daly, M. B., Narod, S. A., Garber, J. E., Lynch, H. T., Weber, B. L., and Brown, M. Modification of *BRCA1*-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat. Am. J. Hum. Genet., *64:* 1371–1377, 1999.

9. Chen, J. J., Silver, D., Cantor, S., Livingston, D. M., and Scully, R. *BRCA1*, *BRCA2*, and *RAD51* operate in a common DNA damage response pathway. Cancer Res., *59* (Suppl.)*:* 1752s–1756s, 1999.

10. Baumann, P., and West, S. C. Role of the human RAD51 protein in homologous recombination and double-stranded break repair. Trends Biochem Sci., *23:* 247–251, 1998.

11. Basile, G., Aker, M., and Mortimer, R. K. Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*. Mol. Cell. Biol., *12:* 3235–3246, 1992.

12. Boice, J. D., Jr., Mandel, J. S., and Doody, M. M. Breast cancer among radiologic technologists. J. Am. Med. Assoc., *274:* 394–401, 1995.

13. Struewing, J. P., Brody, L. C., Erdos, M. R., Kase, R. G., Giambarresi, T. R., Smith, S. A., Collins, F. S., and Tucker, M. A. Detection of eight *BRCA1* mutations in 10 breast/ovarian cancer families, including 1 family with male breast cancer. Am. J. Hum. Genet., *57:* 1–7, 1995.

14. Osborne, R. H., Hopper, J. L., Kirk, J. A., Chenevix-Trench, G., Thorne, H. J., and Sambrook, J. F. kConFab: a research resource of Australasian breast cancer families. Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer. Med. J. Aust., *172:* 463–464, 2000.

15. Hopper, J. L., Chenevix-Trench, G., Jolley, D. J., Dite, G. S., Jenkins, M. A., Venter, D. J., McCredie, M. R., and Giles, G. G. Design and analysis issues in a population-based, case-control-family study of the genetic epidemiology of breast cancer and the Co-operative Family Registry for Breast Cancer Studies (CFR-BCS). J. Natl. Cancer Inst. Monogr., *26:* 95–100, 1999.

16. Apicella, C., Andrews, L., Tucker, K., Bankier, A., Friedlander, M., Venter, D., and Hopper, J. Australian Jewish women's experiences with a breast cancer gene testing program as part of the co-operative family registry for breast cancer studies (CFR-BCS). Am. J. Hum. Genet., *65:* 1048, 1999.

17. Modan, B., Hartge, P., Hirsch-Yechezkel, G., Chetrit, A., Lubin, F., Beller, U., Ben-Baruch, G., Fishman, A., Menczer, J., Ebbers, S. M., Tucker, M. A., Wacholder, S., Struewing, J. P., Friedman, E., and Piura, B. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a *BRCA1* or *BRCA2* mutation. N. Engl. J. Med., *345:* 235–240, 2001.

18. Fishman, A., Dekel, E., Chetrit, A., Lerner-Geva, L., Bar-Am, A., Beck, D., Beller, U., Ben-Baruch, G., Piura, B., Friedman, E., Struewing, J. P., and Modan, B. Patients with double primary tumors in the breast and ovary–clinical characteristics and *BRCA1–2* mutations status. Gynecol. Oncol., *79:* 74–78, 2000.

19. Efron, B., and Tibshirani, R. J. An Introduction to the Bootstrap, Vol. 57, p. 436. New York: Chapman & Hall, 1993.

20. Daniel, W. W. Biostatistics. A Foundation for Analysis in the Health Sciences, pp. 398–401. New York: John Wiley & Sons, Inc., 1983.

21. Bell, D. W., Wahrer, D. C. R., Kang, D. H., MacMahon, M. S., FitzGerald, M. G., Ishioka, C., Isselbacher, K. J., Krainer, M., and Haber, D. A. Common nonsense mutations in RAD52. Cancer Res., *59:* 3883–3888, 1999.

22. Kato, M., Yano, K., Matsuo, F., Saito, H., Katagiri, T., Kurumizaka, H., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Nagawa, H., Nakamura, Y., and Miki, Y. Identification of Rad51 alteration in patients with bilateral breast cancer. J. Hum. Genet., *45:* 133–137, 2000.

23. Bork, P., Blomberg, N., and Nilges, M. Internal repeats in the BRCA2 protein sequence. Nat. Genet., *13:* 22–23, 1996.

24. Levy-Lahad, E., Lahad, A., Eisenberg, S., Dagan, E., Paperna, T., Kasinetz, L., Catane, R., Kaufman, B., Beller, U., Renbaum, P., and Gershoni-Baruch, R. A single nucleotide polymorphism in the *RAD51* gene modifies cancer risk in *BRCA2* but not *BRCA1* carriers. Proc. Natl. Acad. Sci. USA, *98:* 3232–3236, 2001.

25. van der Velden, A. W., and Thomas, A. A. The role of the 5′ untranslated region of an mRNA in translation regulation during development. Int. J. Biochem. Cell. Biol., *31:* 87–106, 1999.

26. Gray, N. K. Translational control by repressor proteins binding to the 5′UTR of mRNAs. Methods Mol. Biol., *77:* 379–397, 1998.